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Effects of testosterone on neuromuscular transmission in rat isolated urinary bladder

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Abstract

Testosterone was examined for its effects on neuromuscular transmission in rat and shrew urinary bladder. In isolated preparations of detrusor muscle from sexually immature male rats (8–10 weeks old) at concentrations of 100–300 μM, it inhibited neuromuscular transmission in a concentration-dependent manner and it also inhibited responses to applied carbachol and diadenosine pentaphosphate (Ap_5A, a P2X receptor agonist). Ethanol (at or above 38 mM), the solvent for testosterone, also caused significant inhibition of neurogenic contractions as well as carbachol- and Ap_5A-induced contractions. In older, sexually mature male rats (over 16 weeks old), testosterone and ethanol had similar effects to those observed in the young male rat, although both were slightly less potent. In young virgin female rats (8–12 weeks old), testosterone and ethanol inhibited neuromuscular transmission; testosterone was approximately 1000 times more potent than in male rats, with a threshold concentration of 30 nM. In the insectivore, *Suncus murinus*, testosterone (0.1 μM–1 mM) caused inhibition of neurogenic and chemogenic responses, but ethanol had no significant effect. Flutamide (50 μM), a genomic testosterone-receptor antagonist, did not inhibit any of the responses to testosterone. It is concluded that testosterone acts predominantly on a postjunctional nongenomic receptor to inhibit urinary bladder detrusor muscle contraction.

Keywords: Autonomic neuromuscular transmission; Nongenomic steroid receptor; (Rat); (*Suncus murinus*); Testosterone; Urinary bladder

1. Introduction

Testosterone and other gonadal steroids have been demonstrated to exert potent effects on autonomic neurones supplying various pelvic targets. For example, testosterone has been shown to exert actions in maintaining the adult features of neurones, such as soma size and transmitter synthesis in noradrenergic neurones. This has been demonstrated by the failure of these neurones to achieve normal size or transmitter levels in castrated rats (Keast and Saunders, 1998). Testosterone is also essential for maintaining neural pathways involved in urine storage and reproductive processes (Keast, 1999). This effect is not universal within these pelvic autonomic nerves as cholinergic neurones to the bladder and bowel do not appear to be affected by testosterone deprivation (Keast, 1999).

The effects of testosterone on innervation in the bladder are chronic and require long-term exposure to testosterone. They are likely to occur according to the classic genomic mechanism of steroidal hormone action involving intracellular receptors, activation of transcription and induction of protein synthesis.

Steroid hormones, including testosterone, can exert effects via mechanisms not accountable for by the classical genomic mechanism. This is due to the rapid onset and lack of modification by protein synthesis inhibitors of these hormonal events. For example, rapid effects of testosterone have been observed in smooth muscle of blood vessels, uterus (Falkenstein et al., 2000) and the vas deferens (Lamas and Spardari, 1993). Furthermore, nongenomic effects on cardiac muscle, osteoblasts and cells of the immune system (Falkenstein et al., 2000) have also been observed.

To date, nongenomic actions of testosterone on neuromuscular transmission in the urinary bladder have not been reported. Thus, the aim of the study was to investigate whether testosterone has an acute action, involving a nongenomic mechanism, on postganglionic efferent neuromus-

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cular transmission in the rat urinary bladder detrusor muscle, which can be regarded as a model of parasympathetic cotransmission (see Hoyle, 1996). Because of the involvement of testosterone in male sexual maturity, the acute effect of testosterone was examined in three groups of rats: young prepubertal males; sexually mature males; young prepubertal females. In addition, because of a report of the lack of an acute effect of testosterone in rabbit urinary bladder (Ratz et al., 1999), the effects of testosterone were examined in the urinary bladder of an animal from a separate order, the house musk shrew, *Suncus murinus*. *S. murinus* is an interesting animal to study because it belongs to the Insectivora, a group that has been classified as being at the stem of the mammalian phylogenetic tree (Colbert, 1958; Novacek, 1992). Furthermore, the characteristics of innervation and neuromuscular transmission in suncus urinary bladder are similar to those of primates, including humans (Hoyle et al., 1998).

2. Materials and methods

2.1. Tissues and protocols

Four groups of animals were used in the investigation: sexually immature male Lister hooded rats (defined by three factors: an age of 8–12 weeks, a weight of 180–334 g, median, 219 g, and on observations of sex organs on dissection); sexually mature Lister hooded rats (age greater than 16 weeks, weight 332–389 g, median 357 g, and appearance of sex organs on dissection); female Lister hooded rats (age 8–12 weeks and weight 184–197 g, median 173 g); adult male *S. murinus* (10–14 months old).

All animals were killed by a rising concentration of CO_2 and cervical dislocation, according to the Home Office Schedule 1 procedure. The bladder was removed and was transected at the level of entry of the ureters, in order to separate the corpus from the trigone.

The bladder was placed in a waxed Petri dish containing cold Krebs' solution. Two lateral cuts were made from the base towards the dome of the bladders in order to make an almost rectangular sheet. The bladder was then pinned down, mucosa uppermost: the mucosa was removed by gentle scraping. The muscularis was then bisected to form two strips of detrusor approximately 2×10 mm. For *Suncus*, the bladders were bisected longitudinally, providing two 'hemi-bladders' (Hoyle et al., 1998). Silk ligatures were attached to the ends of the bladder strips. One was tied to a rigid support placed in a 5-ml sidearm-gassed organ bath containing Krebs' solution maintained at 37 ± 0.5 °C, and the other to an isometric force transducer (Linton). The preparations were suspended vertically between two platinum ring electrodes, 2.5 mm diameter and 10 mm apart that facilitated electrical field stimulation.

The preparations were given an initial load of approximately 0.75 g and then left to equilibrate for approximately 30 min before commencing experimentation. During equilibration, the preparations were stimulated at 8 Hz, using a biphasic square-wave pulse (overall duration 0.3 ms) delivered at a slightly submaximal voltage (40 V) for 10 s every 2 min, using an Experimetria ST-02 stimulator. Data were recorded using an MP100WSW data acquisition system and Acquire software (Biopac).

The Krebs' solution had the following composition (mM): NaCl, 133; KCl, 4.7; $NaHCO_3$, 16.3; NaH_2PO_4, 1.35; $MgSO_4$, 0.6, $CaCl_2$, 2.5 and glucose 7.8, and was gassed with 95% O_2/5% CO_2 giving a pH of 7.3–7.4.

Frequency–response relationships were determined for each preparation by applying electrical field stimulation at 0.5, 1, 2, 4, 8, 16 and 32 Hz, in that order (trains of 10 s, every 2 min). From each bladder, one strip was exposed to testosterone (cumulatively, in the range 1 nM–1 mM). At a given concentration of testosterone, following an equilibration period of at least 10 min, the frequency–response relationship was then redetermined. The second strip from each bladder was used as a time-matched control, and was simultaneously exposed to the same concentration of ethanol as was used as the vehicle for the testosterone as applied to the first strip. P2X receptors were blocked by desensitising the preparation to α,β-methylene ATP (three consecutive applications to give a bath concentration of 30 μM); atropine (1 μM) was added in order to block muscarinic receptors.

In a separate series of experiments, cumulative concentration–response curves were constructed for testosterone, whilst keeping the frequency of electrical field stimulation constant at a frequency of 8 Hz. A minimum of 10 min was allowed between consecutive doses of testosterone (or ethanol in paired preparations). Doses were applied to create an increasing order of final concentration in the organ bath, ranging from 1 to 300 μM for testosterone and 8.7 to 124 mM for ethanol. Responses to electrical field stimulation were analysed in terms of peak contraction (increase in tension above basal tone).

Concentration–response curves were constructed for carbachol (a stable analogue of acetylcholine; 30 nM–100 μM) and P^1,P^5-di(adenosine) pentaphosphate (Ap_5A, a potent agonist of P2X-purinoceptors; 1–100 μM; see Hoyle et al., 2001), by adding a single dose to the organ bath and washing it out after a peak response or a plateau had been observed. A minimum of 10 min was allowed between consecutive concentrations. The concentration–response curve was then repeated on half of the bladder preparations in the presence of testosterone (100 μM), which was reapplied after each washout and left to equilibrate for 10 min before adding each dose of carbachol or Ap_5A. Simultaneously, to the other half of the preparations there was an addition of ethanol (17.4 mM) before the concentration–response curve was repeated, in order to provide a time-matched vehicle control. When experiments were repeated in the presence of flutamide (50 μM), a testosterone receptor antagonist, it was allowed to equilibrate for 30 min.

The neurotoxin, tetrodotoxin (1 μM) a sodium channel blocker, was added to the organ bath in some experiments to confirm that the responses elicited in response to electrical field stimulation were indeed neurogenic.

2.2. Drugs used

All the drugs used in this study were obtained from Sigma. Testosterone was dissolved in ethanol, or a fraction of ethanol, to produce the following stock solutions: 100 mM in 100%, 10 mM in 100%, 1 mM in 50%, 0.1 mM in 20% ethanol. Flutamide was dissolved in 50% ethanol to provide a stock of 50 mM.

2.3. Statistical analysis

Data are presented as mean ± S.E.M. (number of replicates), where the number of replicates also refers to the number of animals. Frequency–response curves and concentration–response curves for carbachol and Ap_5A were compared by two-way analysis of variance with repeated measures; a significant heterogeneity between the curves was demonstrated as $P<0.05$. post hoc Tukey's procedure was used where appropriate, again using $P<0.05$ as the criterion of statistical significance. Mean values of data groups were compared using Student's *t*-test (paired or unpaired as appropriate).

3. Results

3.1. Young male rat

3.1.1. Testosterone concentration–response relationships

Electrical field stimulation (40 V, 0.3 ms, biphasic, 8 Hz, 10 s) evoked contractions that rapidly reached a peak and then declined during the period of excitation.

Ethanol caused significant inhibition of responses to electrical field stimulation at 38 mM and above (Fig. 1). Testosterone (1–300 μM) produced concentration-depend-

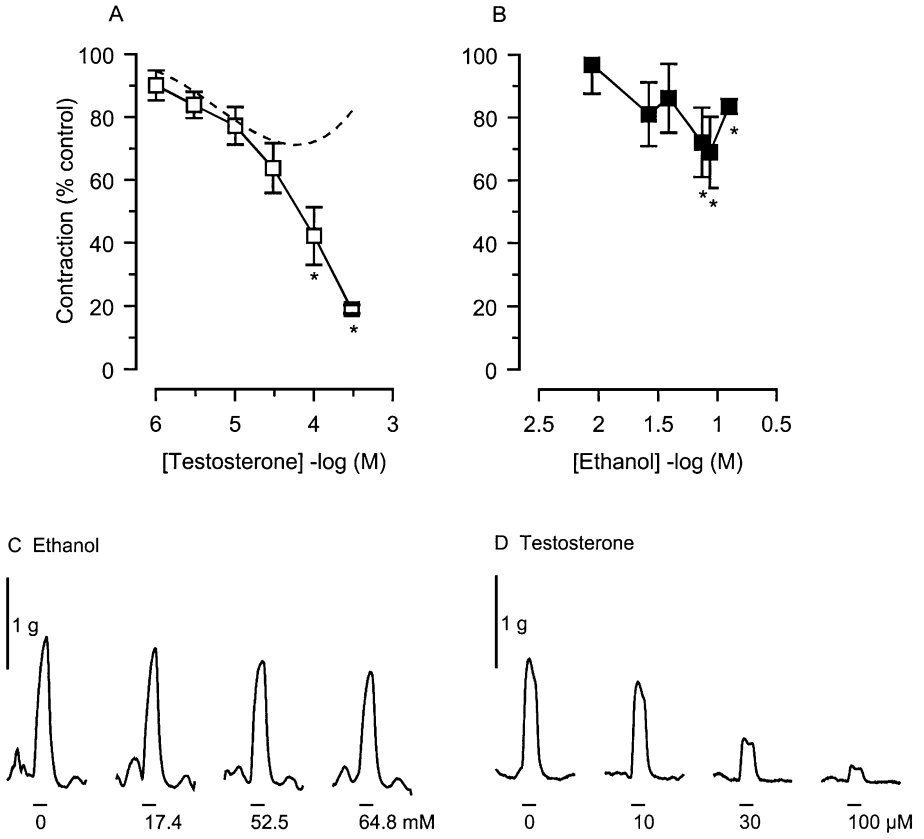

Fig. 1. Effects of testosterone and ethanol on contractions evoked by electrical field stimulation in young male rat urinary bladder detrusor muscle. Electrical field stimulation (8 Hz, 40 V, 0.3 ms, 10 s) evoked contractions. (A) Testosterone (open square, $n=7$) inhibited contractions evoked by electrical field stimulation. Dashed line shows inhibition of contraction due to ethanol, the vehicle for testosterone, interpolated from (B). (B) Ethanol (closed square, $n=7$) inhibited contractions evoked by electrical field stimulation at the three highest concentrations tested (* $P<0.05$, paired Student's *t*-test). Ordinate axes show the peak of contractile responses normalised to a percentage of the control contractions. For (A), significant difference between the inhibition caused by ethanol alone and testosterone, * $P<0.05$, Student's *t*-test. Points show mean ± S.E.M. (C) Application of ethanol caused a small inhibition of contractile responses. (D) Application of testosterone caused a greater concentration-dependent inhibition. The concentrations of ethanol in (C) are the same as those used as the vehicle for the testosterone concentrations in (D). In both panels, the leftmost response was evoked in the absence of ethanol or testosterone.

ent inhibitions of responses to electrical field stimulation (Fig. 1) with a significant inhibition, beyond that due to ethanol, at 100 and 300 μM.

3.1.2. Frequency–response curves

Electrical field stimulation 0.5–32 Hz evoked frequency-dependent contractions. The concentration of ethanol used as a vehicle for 100 μM testosterone provided a bath-concentration of 17.4 mM. At this concentration, ethanol did not have a significant effect on the response evoked at any frequency of stimulation. Testosterone (100 μM) significantly inhibited the contraction at each frequency of stimulation (Fig. 2) but lower concentrations of testosterone had no significant effect.

The frequency–response relationships of the young male rat bladder following desensitisation to α,β-methylene ATP

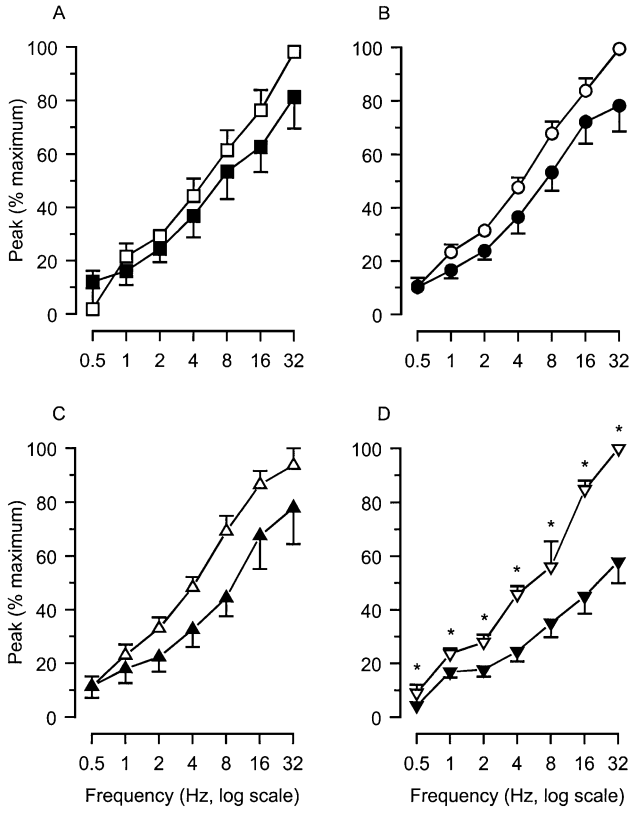

Fig. 2. Effects of testosterone on contractile responses of young male rat urinary bladder detrusor muscle evoked by electrical field stimulation. (A) Responses in the absence (open square, $n=4$), and presence (closed square, $n=4$) of 1 μM testosterone (no significant inhibition, $P>0.05$, two-way ANOVA). (B) Responses in absence (open circle, $n=8$) and presence (closed circle, $n=8$) of 10 μM testosterone (no significant inhibition, $P>0.05$, two-way ANOVA). (C) Responses in the absence (open triangle, $n=4$) and presence (closed triangle, $n=4$) of 30 μM testosterone (no significant inhibition, $P>0.05$, two-way ANOVA). (D) Responses in the absence (open triangle, $n=12$) and presence (closed triangle, $n=12$) of 100 μM testosterone (significant inhibition, $P=0.005$, two-way ANOVA). Ordinate axes show peaks of contractile responses normalised to a percentage of the maximum contractions. Points show mean ± S.E.M., unless occluded by symbol; $*P<0.05$, Tukey's procedure post hoc between pairs of points.

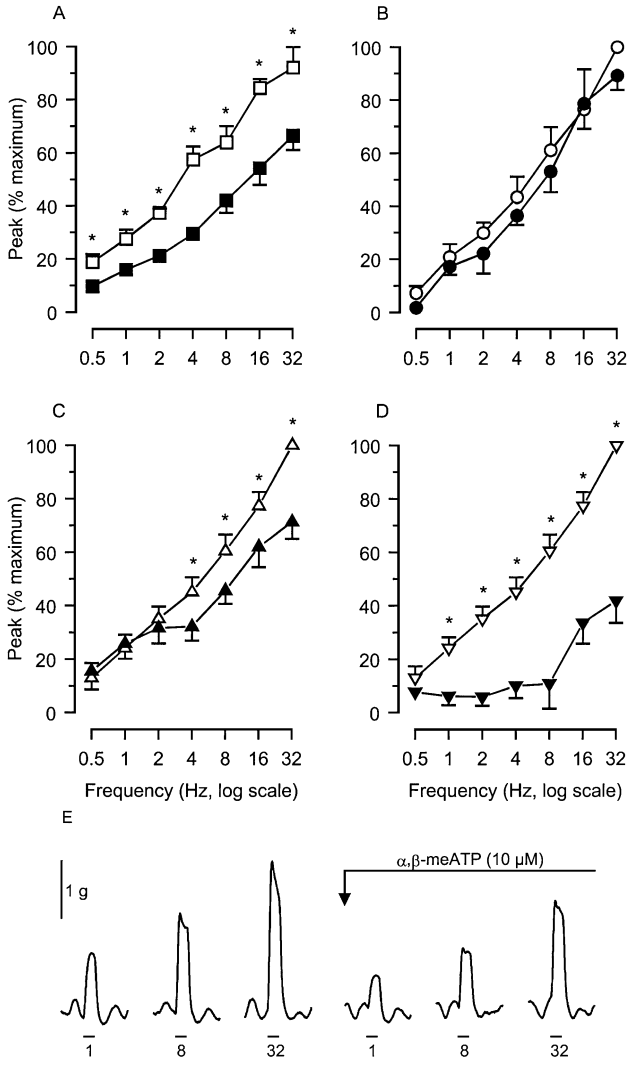

Fig. 3. Effects of testosterone on the contractile responses of young male rat urinary bladder detrusor muscle to electrical field stimulation following desensitisation to α,β-methylene ATP. (A) Responses before (open squares, $n=8$) and after (closed squares, $n=8$) desensitisation to 30 μM α,β-methylene ATP (significant effect, $P<0.001$, two-way ANOVA). (B) Responses following desensitisation to α,β-methylene ATP in the absence (open circles, $n=5$) and presence (closed circles, $n=5$) of 1 μM testosterone. (C) Responses following desensitisation to α,β-methylene ATP in the absence (open triangles, $n=7$) and presence (closed triangles, $n=7$) of 10 μM testosterone (significant effect, $P<0.05$, two-way ANOVA). (D) Responses following desensitisation to α,β-methylene ATP in the absence (open triangles, $n=7$) and presence (closed triangles, $n=7$) of 100 μM testosterone (significant effect, $P<0.05$, two-way ANOVA). Ordinate axes show the peaks of responses normalised to a percentage of the maximum contractions. Points show mean ± S.E.M., unless occluded by a symbol; $*P<0.05$, Tukey's procedure post hoc between pairs of points. (E) Application of α,β-meATP (30 μM) produced a transient contraction (not shown) and subsequently inhibited the contractions at each frequency.

(30 μM, in three applications) showed a significant decrease in the contractile responses (Fig. 3). When P2X receptors were blocked, testosterone inhibited responses to electrical field stimulation at concentrations of 10 μM and above (Fig. 3).

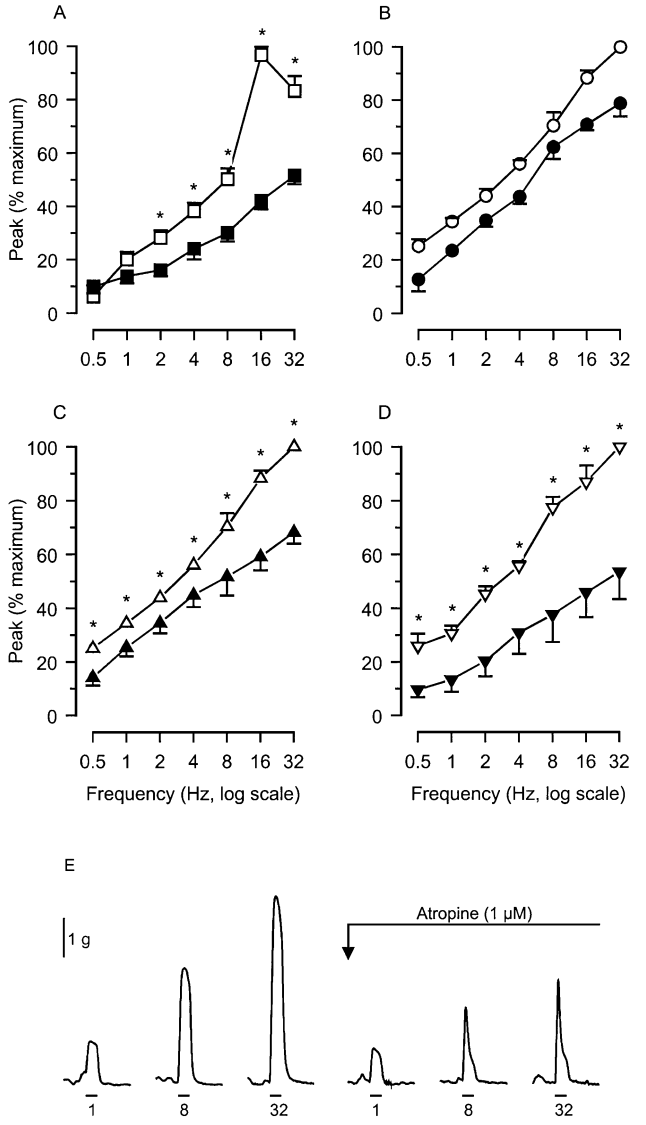

Fig. 4. Effects of testosterone on the contractile responses of young male rat urinary bladder detrusor muscle to electrical field stimulation, in the presence of atropine. (A) Responses in the absence (open squares, $n=4$) and presence (closed squares, $n=4$) of atropine (1 μM). Atropine caused significant inhibition ($P=0.002$, two-way ANOVA). (B) Following the addition of atropine in the absence (open circles, $n=6$) and presence (closed circles, $n=6$) of 1 μM testosterone (no significant inhibition, $P=0.36$, two-way ANOVA). (C) Responses following addition of atropine (1 μM) in absence (open triangles, $n=6$) and presence (closed triangles, $n=6$) of 10 μM testosterone (significant inhibition, $P=0.007$, two-way ANOVA). (D) Responses following addition of atropine in the absence (open triangles, $n=5$) and presence (closed triangles, $n=5$) of 100 μM testosterone (significant inhibition, $P=0.04$, two-way ANOVA). Ordinate axes show the contractile responses normalised to a percentage of the maximum contractions. Points show mean ± S.E.M., unless occluded by symbol; $*P<0.05$, Tukey's procedure, post hoc between pairs of points. (E) Application of atropine (1 μM) inhibited contractions, particularly at higher frequencies.

Atropine (1 μM) significantly reduced the responses to electrical field stimulation (Fig. 4) at frequencies of 2 Hz and above. In the presence of atropine, testosterone at 10 and 100 μM, but not 1 μM, caused significant inhibition at all frequencies (Fig. 4).

3.1.3. Carbachol concentration–response curves

In the young male rat urinary bladder preparations, carbachol (30 nM–100 μM) evoked concentration-dependent contractions that were not significantly affected by ethanol (17.4 mM; Fig. 5). The mean maximum contraction was 3.4 ± 0.54 g ($n=6$).

Testosterone (100 μM) reduced the carbachol-induced contractions (Fig. 5). The shift was significant at all concentrations of carbachol greater than 30 nM ($P<0.05$).

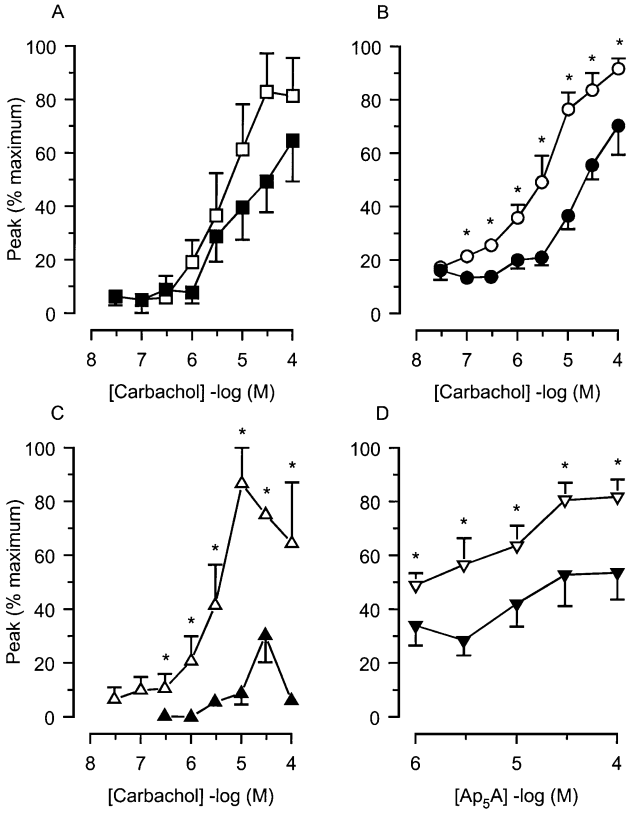

Fig. 5. Effects of testosterone on concentration–response relationships for carbachol and diadenosine pentaphosphate (Ap_5A) in young male rat urinary bladder detrusor muscle. (A) Carbachol in the absence (open squares, $n=7$) and presence (closed squares, $n=7$) of 17.4 mM ethanol (no statistically significant difference, two-way ANOVA). (B) Carbachol in the absence (open circles, $n=7$) and presence (closed circles, $n=7$) of 100 μM testosterone (significant inhibition, $P=0.008$, two-way ANOVA). (C) Carbachol in the presence of flutamide (50 μM, open triangles, $n=4$) and flutamide plus 100 μM testosterone (closed triangles, $n=4$); significant inhibition, $P=0.002$, two-way ANOVA. Ordinate axes show the peaks of responses normalised to a percentage of the maximum contractions. Points show mean ± S.E.M., unless occluded by symbol; $*P<0.05$, Tukey's procedure post hoc between pairs of points. (D) Ap_5A in the absence (open triangles) and presence (closed triangles, $n=7$) of 100 μM testosterone (open triangles, $n=7$); significant effect, $P<0.02$, two-way ANOVA. Points show mean ± S.E.M.; $*P<0.05$, Tukey's procedure post hoc between pairs of points.

3.1.4. AP_5A concentration–response relationships

Ap_5A (1–100 μM) produced concentration-dependent contractions in the young male rat bladder detrusor muscle (Fig. 5). Testosterone (100 μM) produced significant inhibition of Ap_5A-induced responses at all Ap_5A concentrations. The mean maximum contraction evoked by AP_5A was 0.8 ± 0.13 g ($n = 7$).

3.1.5. Flutamide

Flutamide (50 μM) alone had a significant inhibitory effect on the frequency–response relationships (Fig. 6). In the presence of flutamide, the responses following addition of testosterone were significantly lower at all frequencies (Fig. 6). Therefore, there was no antagonism by flutamide on the effects of testosterone on the frequency–response relationship.

In the presence of flutamide (50 μM), testosterone (100 μM) still reduced the effect of carbachol. In fact, there appeared to be a greater inhibition of the contractile responses to carbachol by testosterone in the presence of flutamide (Fig. 5).

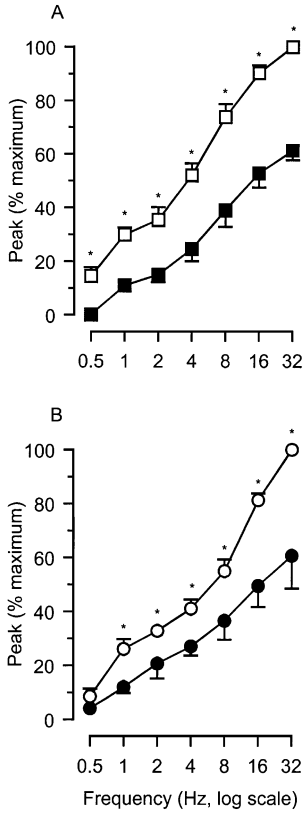

Fig. 6. Effects of flutamide and testosterone on the contractile responses of young male rat urinary bladder detrusor muscle evoked by electrical field stimulation. (A) Responses to electrical field stimulation (8 Hz) in the absence (open squares, $n = 4$) and presence (closed squares, $n = 4$) of 50 μM flutamide. Significant effect, $P < 0.01$, two-way ANOVA. (B) Effects of 100 μM testosterone on responses to electrical field stimulation in the absence (open circles, $n = 4$) and presence of flutamide (closed circles, $n = 4$). Significant effect, $P < 0.05$, two-way ANOVA. Ordinate axes show the responses normalised to a percentage of maximum contractions. Points show mean ± S.E.M., unless occluded by symbol; $P < 0.05$, Tukey's procedure post hoc between pairs of points.

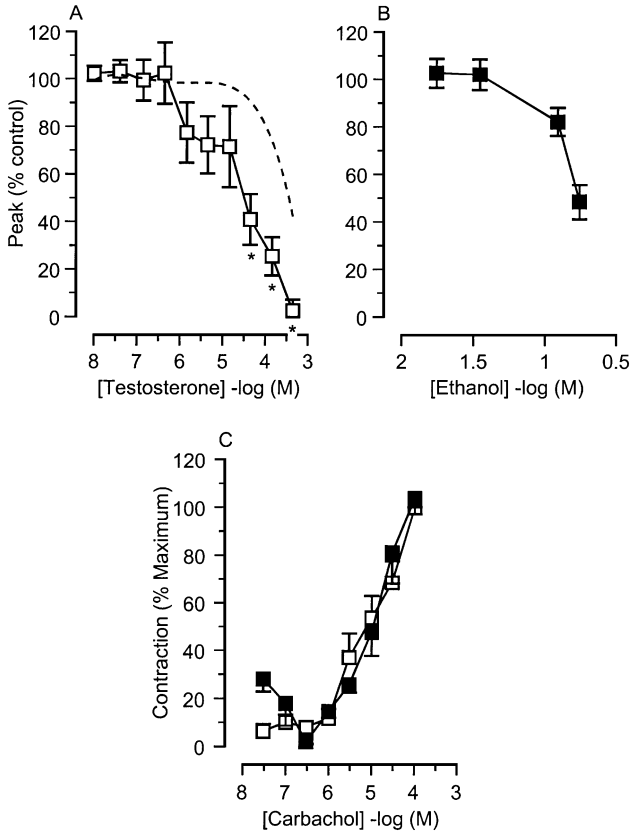

Fig. 7. Testosterone and ethanol concentration–response relationships for mature male rat urinary bladder detrusor muscle. (A) Testosterone (open squares, $n = 4$) caused concentration-dependent inhibition of responses to electrical field stimulation (8 Hz). The dashed line shows the effect of ethanol (the vehicle for testosterone) alone, interpolated from (B). Significant difference from the effect of ethanol alone, * $P < 0.02$, Student's t-test. (B) Ethanol (closed squares, $n = 4$) caused concentration-dependent inhibition of responses to electrical field stimulation ($P = 0.0002$, one-way ANOVA). (C) Concentration–response relationships for carbachol in the absence (open squares, $n = 4$) and presence (closed squares, $n = 4$) of testosterone (100 μM). Testosterone had no significant effect. No significant potentiation exists at 30 nM carbachol in the presence of testosterone ($P = 0.06$, Tukey's procedure post hoc). Ordinate axes show responses normalised to percentage maximum. Points show mean ± S.E.M. unless occluded by symbol.

3.2. Mature male rat

3.2.1. Testosterone concentration–response relationships

Ethanol (17.4 – 174 mM) caused a concentration-dependent inhibition of the responses to electrical field stimulation (40 V, 0.3 ms, biphasic, 8 Hz, 10 s) in the mature male rat urinary bladder (Fig. 7).

Testosterone (10 nM–300 μM) caused a concentration-dependent inhibition of the responses evoked by electrical field stimulation (Fig. 7). The inhibition due to testosterone

was significantly greater than the inhibitory effect of ethanol alone at concentrations above 10 μM. Although testosterone (10–l00 nM) enhanced rather than inhibited contractile responses in some preparations, at concentrations of 1 μM and above it produced inhibitory effects which differed significantly from those seen in the presence of vehicle.

3.2.2. Carbachol concentration–response curves

Carbachol (30 nM–100 μM) produced concentration-dependent contractions of the mature male rat bladder detrusor (Fig. 7). There was no significant difference between the concentration-dependent responses in control conditions from those produced in the presence of testosterone, indicating in this case that testosterone does not appear to have had an inhibitory action on the carbachol-induced contractile responses of the urinary bladder. Although testosterone appeared to potentiate the response to 30 nM of carbachol, this was not statistically significant.

3.3. Young virgin female rat

3.3.1. Testosterone concentration–response relationships

As illustrated in Fig. 8, in the young female rat bladder detrusor ethanol (17.4–348 mM) caused a significant concentration-dependent depression of responses to electrical field stimulation. Depression of the responses occurred at ethanol concentrations above 52.2 mM. Testosterone caused significant concentration-dependent depression of the responses to electrical field stimulation (8 Hz), above that induced by ethanol, at all concentrations above 30 nM.

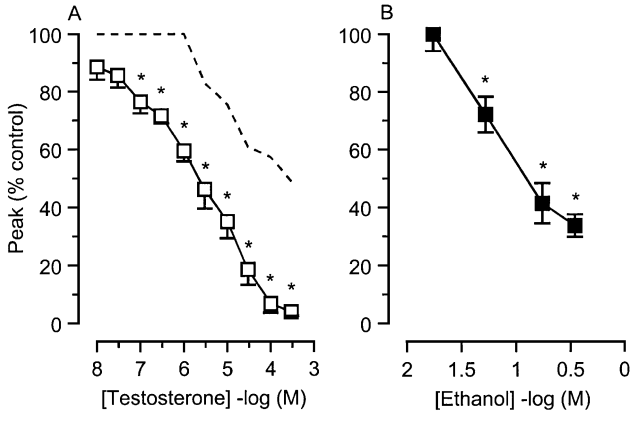

Fig. 8. Effects of testosterone and ethanol on responses to electrical field stimulation in young virgin female urinary bladder detrusor muscle. (A) Testosterone (open squares, $n=5$) caused concentration-dependent inhibition of the peaks of contractions evoked by electrical field stimulation (8 Hz). Dashed line shows the effect of ethanol alone, interpolated from (B). Significant difference between the inhibition caused by ethanol alone and testosterone, $*P<0.05$, Student's t-test. (B) Ethanol (closed square, $n=5$) caused concentration-dependent inhibition of the peaks of responses to electrical field stimulation ($P=0.0001$, one-way ANOVA). Ordinate axes show responses normalised to percentage control contractions. For (B) significant inhibition from the control, $*P<0.05$, Tukey's procedure post hoc. Points show mean ± S.E.M.

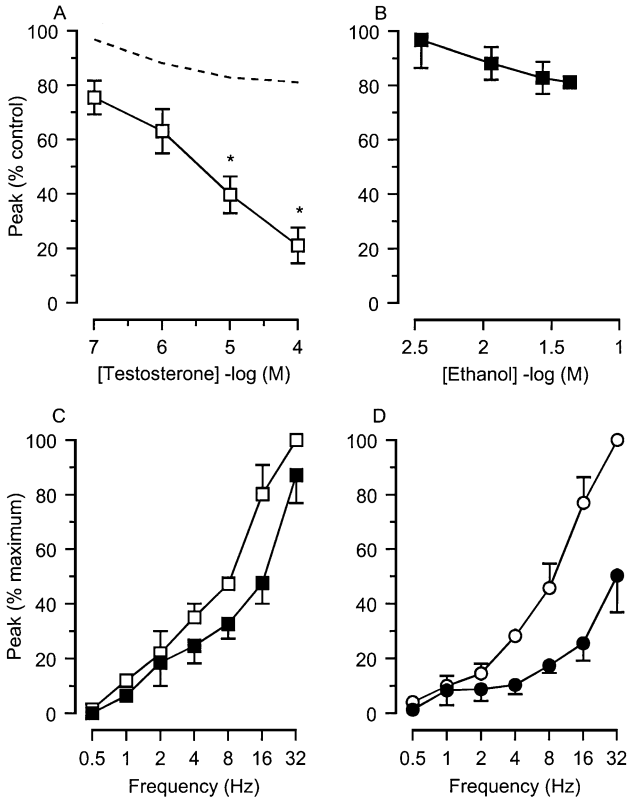

Fig. 9. Effects of testosterone and ethanol on responses to electrical field stimulation in *S. murinus* urinary bladder detrusor muscle. (A) Testosterone (open squares, $n=4$) caused concentration-dependent inhibition of the contractions evoked by electrical field stimulation (8 Hz). Dashed line shows the effect of ethanol alone, interpolated from (B). Statistically significant difference between inhibition caused by ethanol alone and testosterone $*P<0.01$, Student's t-test. (B) Ethanol (closed squares, $n=4$) did not cause concentration dependent inhibition of the peaks of responses to electrical field stimulation ($P=0.42$, one-way ANOVA). Significant difference from the control value $*P<0.05$, Tukey's procedure post hoc. (C) Peaks of contractions to electrical field stimulation in the absence (open squares, $n=3$) and presence (closed squares, $n=3$) of ethanol (17.4 mM). Ethanol had no significant effect ($P>0.05$, two-way ANOVA). (D) Peaks of contractions to electrical field stimulation in absence (open circles, $n=3$) and presence (closed circles, $n=3$) of testosterone (100 μM). Testosterone caused inhibition of responses ($P=0.002$, two-way ANOVA). Statistically significant difference from control values $*P<0.05$, Tukey's procedure post hoc. Ordinate axes show responses normalised to a percentage maximum of the control contractions. Abscissae are on logarithmic scales. Points show mean ± S.E.M.

3.3.2. Flutamide

In the presence of flutamide, electrical field stimulation evoked a frequency-dependent increase in contraction. Testosterone (100 μM) almost abolished the responses to electrical field stimulation in the presence of flutamide (50 μM) in the bladder detrusor muscle in young female rats ($n=5$, data not shown). There was therefore no antagonism of the actions of testosterone by flutamide.

3.4. S. murinus

3.4.1. Testosterone concentration–response relationships

In the *S. murinus* isolated bladder preparations, testosterone (0.1–100 μM) inhibited the responses to electrical field stimulation (8 Hz) in a concentration-dependent manner (Fig. 9). Ethanol (3.48–42.63 mM) did not have a statistically significant effect on the responses to electrical field stimulation.

Testosterone caused significant concentration-dependent inhibition of responses to electrical field stimulation above that produced by ethanol at concentrations greater than 1 μM (Fig. 9).

3.4.2. Frequency–response relationships

Ethanol (17.4 mM) had no significant effect on the responses to electrical field stimulation (0.5–32 Hz, Fig. 9). Testosterone (100 μM) caused significant inhibition of the frequency–response relationships in the *S. murinus* bladder detrusor muscle (Fig. 9).

4. Discussion

This study is the first to show that testosterone has an acute effect on the postganglionic efferent neuromuscular transmission in the urinary bladder detrusor muscle.

Analyses of responses to testosterone were confounded by the inhibitory effects of its vehicle, ethanol, on bladder contractions. The effects of ethanol were concentration-dependent, and became apparent at 38 mM. This is in broad agreement with prior studies that have shown that ethanol has inhibitory actions on bladder contractions in rat and rabbit (Ohmura et al., 1997; Kim et al., 1999).

In the young male rat, testosterone inhibited neuromuscular transmission in the urinary bladder detrusor muscle in a concentration-dependent manner. Inhibition of the responses to electrical field stimulation was apparent at concentrations of testosterone at 100–300 μM. Following either desensitisation to α,β-methylene ATP or treatment with atropine, neurogenic contractions were significantly inhibited by testosterone. Thus, both the cholinergic and the purinergic components of neuromuscular transmission were affected.

The inhibitory effect of testosterone on the detrusor contractions induced by carbachol indicates a postjunctional action. If the inhibition were purely due to testosterone acting prejunctionally then no inhibition of responses to applied substances by testosterone would have occurred. Nevertheless, it cannot be ruled out that testosterone does also have prejunctional actions, contributing to the overall action of inhibiting contractile responses.

Testosterone like other steroid hormones, according to the traditional model, binds to intracellular receptors, subsequently modulating transcription and protein synthesis, thus triggering genomic events finally responsible for effects with a relatively long latency (Falkenstein et al., 2000). However, due to the very rapid effects that testosterone exhibited in exerting its actions on the contractility of the young rat male bladder, it is clear that these actions are incompatible with the genomic model. In experiments where chronic effects of testosterone on autonomic nerves have been demonstrated (Keast and Saunders, 1998), in which withdrawal of testosterone results in decreased soma size of nerves supplying the bladder, the actions of testosterone in maintaining soma size could be accountable for by genomic actions. Not only are those effects produced over a much longer time-course but also the sites of genomic activity, i.e. the cell bodies, were intact. In the present study, there would be no, or few, nerve cell bodies present in the bladder preparations, since the major pelvic ganglia lie in the trigone area, and thus no perikaryal steroidal binding sites would have been available for the testosterone.

Flutamide did not inhibit responses to testosterone. This also suggests the existence of a nongenomic mechanism for the action of testosterone in the rat urinary bladder since flutamide is a genomic testosterone receptor antagonist (Neri et al., 1972; Peets et al., 1974).

The present study does not provide information about the exact location, or mechanism by which testosterone carried out its action, although it does indicate that these rapid, nongenomic actions of testosterone appeared to be occurring predominantly at a postjunctional site. Since testosterone inhibits cholinoceptor- and purinoceptor-mediated contractions of the bladder its action is likely to be in a pathway common to both the receptors.

Investigations involving the rat superior cervical ganglion (Gejman and Cardinali, 1983) have illustrated the ability of testosterone to depress muscarinic binding sites, assessed by [^{3}H]-quinuclydinyl benzylate binding, due to actions on the receptor, and modifications in affinity of muscarinic binding due to testosterone have also been reported in the hypothalamic preoptic area (see Gejman and Cardinali, 1983). At the muscarinic M3 receptor in the rat urinary bladder (Longhurst et al., 1995), similar effects could occur.

Testosterone has been demonstrated to decrease influx of calcium ions in vascular and uterine smooth muscle (Gutiérrez et al., 1994; Crews and Khalil, 1999), and also in vascular smooth muscle it has been shown to increase the efflux of potassium ions resulting in hyperpolarisation (Costarella et al., 1996). These mechanisms remain to be investigated in the urinary bladder.

Results from the urinary bladder detrusor muscle of mature male rats were similar to those from the young male rats. Both testosterone and ethanol inhibited contractile responses evoked by electrical and chemical stimuli. However, there were some differences. In the mature male rats, there was potentiation of contraction in the detrusor muscle in response to electrical field stimulation at the lowest concentrations of testosterone, whereas in the young male rats there was no potentiation at all of contractile responses

by testosterone. Potentiation occurred at some higher concentrations of testosterone also; not all preparations showed this effect but it is reflected in the relatively large standard errors of the mean values.

Young virgin female rats also produced results that were generally consistent with those for the young male rat. However, in the female rat testosterone inhibited responses to electrical field stimulation at concentrations as low as 30 nM. In both young and mature male rats, testosterone concentrations required to produce significant inhibition of responses to electrical field stimulation (8 Hz), were much higher, and were around 100 to 300 μM.

The substantial difference between the potencies of testosterone in the young females and young males is somewhat unusual. In many cases, testosterone sensitivity is similar between the two sexes until the males reach puberty, when subsequently their sensitivity to testosterone increases. For example, in aortic smooth muscle testosterone inhibits contractile responses evoked by activation of α-adrenoceptors, with a greater potency in adult male or castrated adult male rats than in normal female rats (Crews and Khalil, 1999).

It has previously been shown that in the rabbit, a lagomorph, acute application of testosterone does not inhibit contractions of urinary bladder detrusor muscle (Ratz et al., 1999). However, in *S. murinus,* an Insectivore, classified as being at the stem of the mammalian phylogenetic tree (Colbert, 1958; Novacek, 1992), the inhibition of the urinary bladder detrusor in response to testosterone was broadly similar to that of the male rats. There may have been a greater sensitivity to testosterone in this species than in the rat as the threshold concentration of testosterone for inhibiting responses to electrical field stimulation was approximately 10-fold lower. Ethanol did not cause significant inhibition of responses to electrical field stimulation in *S. murinus*. This was in contrast to all the groups of rats.

Although to extrapolate these findings from small animals to humans would be unreliable, it is likely that similar mechanisms are found in humans. The implication of these findings is that testosterone would inhibit bladder emptying, thus increasing urinary retention. Thus, activation of testosterone receptors in the urinary bladder may prove to be therapeutically useful in treating forms of urinary incontinence. These include overactive bladder conditions caused by elevated muscarinic and purinergic receptor activation, e.g. interstitial cystitis (Palea et al., 1993), and also in detrusor reflex dysynergia, as in spinal cord injury.

In conclusion, testosterone has been demonstrated to have an acute, nongenomic action on urinary bladder detrusor neuromuscular transmission, ultimately inhibiting contraction of the muscle in the male and female rat and *S. murinus* urinary bladder. This is dependent on the concentrations of testosterone being above approximately 100 μM in male rats and 30 nM in female rats. The effect of testosterone in inhibiting neuromuscular transmission is most likely to be predominantly a postjunctional action, involving components of the contractile protein system and possibly calcium ion movement.

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